BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

British Journal of Haematology, 1997, 99, 245–256

Review

AMYLOIDOSIS: A REVIEW OF RECENT DIAGNOSTIC AND THERAPEUTIC DEVELOPMENTS

Summary

Amyloid deposition is associated with a diverse range of disorders that includes Alzheimer's disease, type II diabetes mellitus and dialysis arthropathy. Although less common, systemic AA and AL amyloidosis remain important because effective treatments have increasingly become available.

The pathology in all forms of amyloidosis involves the extracellular deposition of protein as characteristic fibrillar aggregates which interfere with tissue structure and function. Amyloid fibrils are derived from different unrelated proteins in the different forms of the disease but share many common properties, including the capacity to bind the normal plasma protein serum amyloid P component (SAP). This is the basis for our development of radiolabelled SAP as a nuclear medicine tracer for the diagnosis and quantitative monitoring of amyloid. Serial studies have shown that the deposits are far from inert but are actually turned over quite rapidly in many patients.

The treatment of amyloidosis involves supportive measures whilst every effort is made to reduce the supply of the respective fibril precursor protein. Under favourable circumstances further amyloid deposition will be prevented, existing deposits will regress and improvement of organ function will occur. Since this strategy is not always possible or may fail, new approaches to inhibit fibril formation and promote regression of amyloid are being pursued.

Introduction

The amyloidoses are a heterogenous group of disorders characterized by the extracellular deposition of protein in an abnormal fibrillar form (Pepys, 1995), with pathognomonic tinctorial properties. Amyloidosis may be hereditary or acquired, and the deposits may be focal, localized or systemic in distribution. Focal or small amounts of amyloid may be incidental, particularly in the elderly, whereas systemic amyloidosis is progressive and usually fatal.

Amyloid deposits are composed largely of protein fibrils, the peptide subunits of which differ in the different forms of the disease and constitute the basis for the classification of the clinical amyloidoses (Tables I and II) (Husby, 1992). All types of amyloid share a remarkably similar core structure of β-sheets with strands perpendicular to their long axis (Booth *et al*, 1997), despite marked heterogeneity in sequence and tertiary folding of the various precursor proteins.

Amyloid deposits are rich in glycosaminoglycan (GAG) material, in the form of heparan sulphate and dermatan sulphate, which is non-covalently bound to the amyloid fibrils (Nelson *et al*, 1991). The precise role of GAGs in amyloid remains unclear, but there is experimental evidence to suggest that they may have fibrillogenic effects on certain amyloid fibril precursor proteins. Another universal constituent of amyloid deposits is amyloid P component (Baltz *et al*, 1986), derived from and identical to serum amyloid P component (SAP), a remarkably stable and proteinase resistant, circulating glycoprotein (Pepys *et al*, 1994). SAP binds to amyloid fibrils in a calcium dependent manner, and may contribute to the relative stability of amyloid deposits *in vivo*. The development of scintigraphic imaging using radiolabelled SAP as an agent to specifically target amyloid deposits has lately provided a wealth of information on the natural history and response to therapy of the systemic amyloidoses.

A diagnosis of amyloidosis must always be followed up by characterization of the fibril protein type to determine the appropriate management. At present, no treatment specifically causes the mobilization of amyloid but recent observations in several types of amyloidosis have revealed that measures which reduce the supply of the respective amyloid fibril precursor protein, frequently result in major regression of deposits. This indicates that amyloidosis is a dynamic process (Hawkins *et al*, 1993a). Although this type of approach in conjunction with supportive therapies has been extremely successful in many patients with systemic amyloidosis, new treatments that inhibit the formation, persistence and effects of amyloid deposits are still required.

Pathogenesis of amyloidosis

The formation, deposition and persistence of amyloid fibrils necessitate the production of the respective fibril precursor protein. The primary structure of fibril precursor proteins is undoubtedly a major determinant of their amyloidogenicity, and although whole intact precursor molecules occasionally form the amyloid fibrils *in vivo* (Saraiva *et al*, 1984; Gorevic *et al*, 1986), they are usually composed of fragments that have undergone partial proteolytic cleavage (Glenner, 1980a, b). The exact timing of proteolytic cleavage with respect to fibril formation is not known. Furthermore, the reasons why amyloid is deposited in certain individuals and not others with apparently identical precursor protein supply, the factors governing anatomical distribution, rate of onset and progression, and the clinical effects of amyloid deposits, are not understood. In the experimental murine model of amyloidosis, in which mice given inflammatory stimuli for 3–6 weeks develop AA amyloid, the latent period can be reduced to 2 days in animals that have received a

Correspondence: Dr J. D. Gillmore, Immunological Medicine Unit, Department of Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 0NN.

Table I. Acquired amyloidosis syndromes.

Clinical syndrome	Fibril precursor
Systemic AL amyloidosis, associated with immunocyte dyscrasia, myeloma, monoclonal gammopathy, occult dyscrasia	AL fibrils derived from monoclonal immunoglobulin light chains
Local nodular amyloidosis (skin, respiratory tract, urogenital tract, etc.) associated with focal immunocyte dyscrasia	AL fibrils derived from monoclonal immunoglobulin light chains
Reactive systemic AA amyloidosis, associated with chronic active diseases	AA fibrils derived from serum amyloid A protein (SAA)
Senile systemic amyloidosis	Transthyretin (TTR) derived from plasma TTR
Focal senile amyloidosis: atria of the heart	Atrial natriuretic peptide
brain	β-protein
joints	Not known
seminal vesicles	Seminal vesicle exocrine protein
prostate	β2-microglobulin
Non-familial Alzheimer's disease, Down's syndrome	β-protein derived from β-amyloid protein precursor (APP)
Sporadic cerebral amyloid angiopathy	β-protein derived from β-amyloid protein precursor (APP)
Transmissible spongiform encephalopathies; prion diseases	Prion protein (PrP) derived from prion protein precursor
Type II diabetes mellitus	Islet amyloid polypeptide (IAPP), amylin, derived from its precursor protein
Endocrine amyloidosis, associated with APUDomas	Peptide hormones or fragments thereof (e.g. precalcitonin in medullary carcinoma of the thyroid)
Dialysis-related amyloid	β_2-microglobulin derived from high plasma levels
Primary localized cutaneous amyloid	? Keratin derived
Ocular and orbital amyloid	Not known

single injection of an extract of amyloidotic tissue. The nature and mode of action of this so-called 'amyloid enhancing factor' (AEF) (Axelrad *et al*, 1982) remain poorly characterized but may account for some of the marked individual variation in susceptibility to fibril formation.

The natural history of amyloidosis is that deposits usually accumulate inexorably. The fact that amyloid deposits regress when the supply of fibril precursors is reduced, indicates that deposits are in a state of dynamic turnover. In the absence of treatment the rate of amyloid deposition evidently usually exceeds the capacity for its mobilization.

Amyloid deposits probably exert much of their pathological effects through their physical presence, causing disruption of normal tissue architecture. It is possible, however, that amyloid fibrils are cytotoxic, for example by inducing apoptosis, and that some of their effects in conditions such as prion diseases, in which deposits are scanty, are mediated in this way (Lorenzo *et al*, 1994).

Clinical amyloidosis syndromes

Reactive systemic, AA, amyloidosis. The AA protein, which forms AA amyloid fibrils, is derived from the circulating acute phase reactant serum amyloid A (SAA) by proteolytic cleavage. SAA is an apolipoprotein of high-density lipoprotein (HDL). It is synthesized by hepatocytes under transcriptional regulation by cytokines such as IL1, IL6 and TNF, and the normal plasma concentration of 10 mg/l may increase to over 1000 mg/l within 24 h of an acute stimulus. Sustained, high circulating levels of SAA are a prerequisite for the development of AA amyloidosis, but the reason that only a small proportion of patients with a persistent acute phase response develop amyloid remains unclear.

Any pathological process resulting in a sustained acute phase response, may therefore be complicated by AA amyloidosis (Table III). The commonest causes in the Western World are idiopathic inflammatory rheumatic diseases (Gertz & Kyle, 1991), which are typically present for 8–14 years before amyloid is diagnosed. In the U.K. 5–10% of patients with rheumatoid arthritis or juvenile chronic arthritis (JCA) are affected. Amyloidosis is exceptionally rare in systemic lupus erythematosus and ulcerative colitis, reflecting the modest acute phase response evoked by these particular conditions.

AA amyloid deposition may be extensive without causing symptoms (Hawkins *et al*, 1993b). The most common mode of presentation is with non-selective proteinuria, nephrotic syndrome and/or renal insufficiency. Acute renal failure may be precipitated by minor insults, and rarely amyloid deposits give rise to haematuria, tubular defects, and diffuse renal

Table II. Hereditary amyloidosis syndromes.

Clinical syndrome	Fibril protein
Predominant peripheral nerve involvement, familial amyloid polyneuropathy (FAP); autosomal dominant	Transthyretin (TTR) genetic variants (most commonly Met30, but over 55 others described)
Predominant peripheral nerve involvement, familial amyloid polyneuropathy (FAP); autosomal dominant	Apolipoprotein A1 (apoA1) N-terminal fragment of genetic variant Arg26
Predominant cranial nerve involvement with lattice corneal dystrophy; autosomal dominant	Gelsolin, fragment of genetic variants Asn187 or Tyr187
Non-neuropathic, prominent visceral involvement (Ostertag-type); autosomal dominant	ApoA1, N-terminal fragment of genetic variants Arg26, Arg50, Arg60, etc.
Non-neuropathic, prominent visceral involvement (Ostertag-type); autosomal dominant	Lysozyme genetic variant Thr56 or His67
Non-neuropathic, prominent visceral involvement (Ostertag-type); autosomal dominant	Fibrinogen α-chain, fragment of genetic variants, Leu554 or Val526
Predominant cardiac involvement, no clinical neuropathy; autosomal dominant	TTR genetic variants Thr45, Ala60, Ser84, Met111, Ile122
Hereditary cerebral haemorrhage with amyloidosis (cerebral amyloid angiopathy); autosomal dominant: Icelandic type	Cystatin C, fragment of genetic variant Glu68
Dutch type	β-protein derived from genetic variant APP Gln693
Familial Alzheimer's disease	β-protein derived from genetic variant APP Ile717, Phe717 or Gly717
Familial dementia; probable Alzheimer's disease	β-protein derived from genetic variant APP Asn670, Leu671
Hereditary spongiform encephalopathies, prion diseases	Prion protein (PrP) derived from genetic variants of PrP precursor protein 51–91 insert, Leu102, Val117, Asn178, Lys200
Familial Mediterranean fever, prominent renal involvement; autosomal recessive	AA derived from SAA
Muckle-Well's syndrome, nephropathy, deafness, urticaria, limb pain	AA derived from SAA
Cardiomyopathy with persistent atrial standstill	Not known
Cutaneous deposits (bullous, papular, pustulodermal)	Not known

calcification. End-stage renal failure is the cause of death in 40–60% of cases. Patients sometimes present with organomegaly, e.g. hepato/splenomegaly, occasionally without accompanying renal dysfunction, but renal deposits are invariably present in such cases nevertheless. The spleen is always affected early, and functional hyposplenism may eventually develop. The adrenal glands are involved in at least one third and the liver is infiltrated in a quarter of cases. Although function in these organs is typically preserved, liver involvement is a sign of extensive disease and is associated with a poor prognosis. Clinical sequelae from the frequent histological cardiac and gut involvement, are unusual (Pepys, 1995).

Systemic amyloidosis associated with immunocyte dyscrasia, AL amyloidosis. AL amyloid fibrils are derived from the N-terminal region of monoclonal immunoglobulin light chains, more commonly λ than κ, and consist of the whole or part of the variable (V_L) domain, although occasionally intact light chains are present. Sequence analysis of Bence-Jones proteins suggests that 'amyloidogenic' light chains have specific amino acid substitutions compared to 'non-amyloidogenic' light chains.

AL amyloidosis can complicate most clonal B-cell dyscrasias, including up to 15% of patients with multiple myeloma and a much smaller proportion of those with lymphomas, macroglobulinaemia and otherwise 'benign' monoclonal gammopathies. However, the prevalence of subtle monoclonal gammopathies is sufficiently high so that these dyscrasias account for up to 80% of AL amyloidosis overall. A monoclonal component is present in the serum or urine of 65% and 86% respectively, but in 14% of patients with AL amyloid the underlying gammopathy cannot be characterized.

Clinical manifestations of systemic AL amyloidosis are protean, and almost any organ other than the brain can be directly involved. The heart is histologically involved in most cases, with symptomatic restrictive cardiomyopathy the presenting feature in up to one third of cases, and the cause of death in one half (Kyle *et al.* 1986). Renal AL deposits have the same effect as renal AA amyloid. Gut

Table III. Conditions associated with AA amyloidosis.

Chronic inflammatory disorders
Rheumatoid arthritis
Juvenile chronic arthritis
Ankylosing spondylitis
Psoriasis and psoriatic arthropathy
Reiter's syndrome
Adult Still's disease
Behçet's syndrome
Crohn's disease
Chronic microbial infections
Leprosy
Tuberculosis
Bronchiectasis
Decubitus ulcers
Chronic pyelonephritis in paraplegics
Osteomyelitis
Whipple's disease
Malignant neoplasms
Hodgkin's disease
Renal carcinoma
Carcinoma of gut, lung, urogenital tract
Basal cell carcinoma
Hairy cell leukaemia

involvement can cause motility disturbances (also caused by autonomic neuropathy), malabsorption, perforation, haemorrhage, or obstruction. Hyposplenism, occasionally with splenomegaly, may be present. Carpal tunnel syndrome occurs in 40% and peripheral sensorimotor neuropathy in 20%, often in association with autonomic neuropathy. Skin involvement takes the form of papules, nodules and plaques, usually on the face and upper trunk and involvement of the dermal blood vessels results in purpura characteristically around the eyes. Macroglossia is less frequent, but almost pathognomonic. Articular AL amyloid may cause an asymmetric arthropathy. Deficiency of factor X and occasionally factor IX may cause a bleeding diathesis.

Hereditary systemic amyloidosis. Hereditary systemic amyloidosis is extremely rare but exists in many forms. It is due to autosomal dominant inheritance of variant amyloidogenic proteins. The first of these proteins to be identified (Costa *et al*, 1978), and the most common cause of hereditary amyloidosis, is variant transthyretin (TTR), usually resulting in the clinical syndrome of familial amyloidotic polyneuropathy (FAP). 59 mutations in the TTR gene have now been reported to cause amyloidosis (Benson & Uemichi, 1996; unpublished data). TTR is produced predominantly in the liver, and is involved in the transport of thyroid hormones and vitamin A. Normal wild-type TTR has a β-sheet structure, with inherent amyloidogenicity (see senile systemic amyloidosis below), and little change is required for this property to be greatly enhanced.

The clinical syndrome of FAP is characterized by progressive peripheral and autonomic neuropathy with varying degrees of visceral amyloid deposition in the spleen, heart, vitreous of the eyes, thyroid and adrenal glands. Amyloidotic cranial neuropathy with systemic involvement, first reported and most prevalent in Finland, results from mutations in the gene encoding the protein gelsolin (Maury, 1991).

Mutations in the apolipoprotein AI (Nichols *et al*, 1990; Soutar *et al*, 1992), lysozyme (Pepys *et al*, 1993) and α-fibrinogen (Benson *et al*, 1993) genes are associated with hereditary non-neuropathic systemic amyloidosis. Most patients present with hypertension and renal failure, although the heart, spleen, liver, bowel, connective tissue and exocrine glands may all be involved to varying degrees.

Dialysis related amyloidosis (DRA). β_2-microglobulin (β_2M) is the light chain of class I MHC antigens, is present on all nucleated cells, and forms the amyloid fibrils in DRA. Persistently elevated levels of β_2M, resulting from its failure to pass the dialysis membrane, is universal in dialysed patients, and is a prerequisite for the development of DRA. DRA affects most patients on long-term haemodialysis (Gejyo *et al*, 1985), and has been reported in patients receiving continuous ambulatory peritoneal dialysis (Gagnon *et al*, 1986). The amyloid deposits are predominantly found in the joints, bones and periarticular tissues resulting in arthralgia, carpal tunnel syndrome and bone cysts, but occasionally more extensive deposition occurs and deaths associated with systemic β_2M amyloidosis have been reported.

Senile systemic amyloidosis. Up to 25% of elderly individuals have clinically silent systemic deposits of normal wild-type TTR. Occasionally, more extensive deposition in the heart causes cardiac failure and may be fatal.

Localized amyloidosis. Amyloid deposits localized to particular organs occur in a wide variety of different forms and presumably reflect either local production of fibril precursors, or the properties of the particular microenvironment, resulting in localization and fibril formation of systemically distributed precursor proteins. The most common clinically significant forms of local amyloid occur in the skin, respiratory or genital tracts and are of AL type associated with local monoclonal B cell or plasma cell proliferation. Surgical resection of these localized 'amyloidomas' may be curative.

Cerebral amyloidosis. The brain and intracerebral blood vessels are rarely affected in the systemic amyloidoses but are important sites for local deposition of amyloid (Duchen, 1992). The most frequent and important type of cerebral amyloid is that related to Alzheimer's disease, which is the commonest cause of dementia worldwide. The fibril protein in the intracerebral and cerebrovascular amyloid of Alzheimer's disease, Down's syndrome and hereditary amyloid angiopathy of Dutch type is known as β-protein. It is a 39–43 residue sequence derived from β-amyloid precursor protein (APP). How the β-protein fragment *per se*, or the amyloid fibrils which it forms contribute to neuronal dysfunction is controversial, but the fact that APP mutations can cause Alzheimer's disease with the same neuropathological features as sporadic cases (Wisniewski *et al*, 1985) suggests that the APP and β-protein pathway is of primary pathogenetic importance. The recently discovered mutations

in presenilin 1 and 2 proteins that cause most cases of familial Alzheimer's disease are associated with increased production of the most amyloidogenic form of β-protein ($A\beta_{1-42}$) (Scheuner *et al*, 1996), further strengthening the link between cerebral amyloidosis and pathogenesis of neurodegeneration.

Diagnosis

Histology. The diagnosis of amyloidosis usually requires histological confirmation. Amyloid stained with Congo red gives pathognomonic red–green birefringence, when viewed under crossed polarized light, and this property remains the diagnostic gold standard (Puchtler *et al*, 1962).

Biopsy of an affected major organ, for example the kidney or heart, is usually diagnostic but less invasive alternatives in systemic amyloidosis include fine needle aspiration of subcutaneous fat (Westermark & Stenkvist, 1973), and rectal or labial salivary gland biopsy (Delgado & Mosqueda, 1989), all of which can produce positive results in up to 80% of cases.

Adequate tissue specimens and proficient histological technique are necessary to maintain high diagnostic sensitivity and specificity. Congo red histology should always be followed by immunohistochemical staining of tissue to determine the amyloid type; other procedures such as Congo red staining after permanganate treatment no longer have a useful place in amyloid typing. Antibodies against most known amyloid fibril proteins are available, but in AL amyloidosis diagnostic results are obtained in only about half of cases because fibril light chain fragments contain unique epitopes that are often not recognized by standard antisera to κ or λ chains. Electron microscopy alone is insufficient for confirmation of the diagnosis of amyloidoisis.

SAP scintigraphy. Radiolabelled SAP rapidly and specifically localizes to amyloid deposits *in vivo* in proportion to the quantity of amyloid present, allowing diagnosis and quantification of deposits by scintigraphy (Hawkins *et al*, 1988a, b; 1990). The technique has almost 100% diagnostic sensitivity in systemic AA amyloidosis, and approximately 90% in AL type and is the only method available for serially monitoring amyloid throughout the body. It has enabled important observations regarding amyloid to be made, including the different patterns of distribution in the different forms of the disease, e.g. Fig 1, the demonstration of amyloid in sites not normally available for biopsy, and the poor correlation between quantity of amyloid and degree of organ dysfunction. In addition, scintigraphy has permitted the study of the natural history of the disease and effects of treatment (Holmgren *et al*, 1993; Hawkins *et al*, 1994a, b).

Biopsy histology and SAP scintigraphy remain complementary techniques. SAP scintigraphy allows non-invasive, macroscopic, whole body imaging, whereas histology is more sensitive for the demonstration of microscopic deposits. Although SAP scintigraphy is routinely performed at Hammersmith Hospital, London, and a few other centres, it is not yet available generally.

Other investigations. Cardiac amyloid is poorly visualized by SAP scintigraphy, but ECG and echocardiography in combination are diagnostically sensitive, providing additional functional and prognostic information. In patients with cardiac amyloid infiltration the ECG commonly shows small voltages and pathological 'Q' waves (pseudo-infarct pattern) in the anterior chest leads. Two-dimensional echocardiography classically reveals small, concentrically hypertrophied ventricles with 'sparkling' echodensity.

Fig 1. ^{123}I-SAP scintigraphy in two young adults with systemic amyloidosis. On the left is an anterior whole body scan of a 26-year-old man showing uptake of tracer into substantial liver, spleen and bone marrow amyloid, a distribution diagnostic of systemic AL amyloidosis; on the right is a posterior whole body scan showing AA amyloid deposits in the spleen, adrenals and kidneys of a 34-year-old woman with rheumatoid arthritis. The presence and type of amyloid were corroborated histologically in both cases.

Any disorder associated with amyloidosis should be sought and characterized, such as a B-cell dyscrasia in AL amyloidosis or the cause of the acute-phase response in AA amyloidosis. In approximately 15% of patients with AL amyloidosis, the underlying B-cell clone is not demonstrable on bone marrow examination or through evidence of a monoclonal product in serum or urine. In a proportion of these cases the clonal disorder can be identified by detecting immunoglobulin gene rearrangements, using Southern blotting or polymerase chain reaction (PCR) techniques (Vigushin *et al*, 1994b).

When hereditary amyloidosis is suspected, the gene defect should be sought and, once identified, screening and counselling offered to relatives.

Ultimately, some cases of amyloid can be characterized only after fibrils have been isolated from amyloidotic tissue and subjected to amino acid sequencing.

Management

The search continues for a treatment that specifically causes mobilization of amyloid deposits. At present however, the aim of therapy is to reduce the supply of amyloid precursor

Table IV. Reducing the supply of fibril precursors in systemic amyloidosis.

Disease	Aim of treatment	Example of treatment
AA amyloid	Suppress acute phase response	Immunosuppression in rheumatoid arthritis, colchicine in FMF
AL amyloid	Suppress production of monoclonal light chains	Chemotherapy for myeloma and monoclonal gammopathy
Hereditary amyloidosis	Eliminate source of genetically variant protein	Orthotopic liver transplantation for variant transthyretin-associated FAP
Dialysis-related amyloidosis	Reduce plasma concentration of β_2M	Renal transplantation

proteins, in the hope of arresting progression of the disease (Table IV). Few clinical trials have been performed and the approach to treatment remains somewhat empirical. This approach, however, has resulted in preservation of organ function, and improved survival in many patients (Tan *et al*, 1995). Speculation that existing amyloid deposits may regress under these circumstances has now been systematically confirmed using SAP scintigraphy, in several types of amyloidosis, e.g. Fig 2 (Hawkins *et al*, 1993b, 1994c; Holmgren *et al*, 1993; Tan *et al*, 1996), but this generally takes months or years.

Supportive therapy, in order to delay target organ failure, maintain quality of life and prolong survival is vital, whilst therapy aimed at the underlying defect has time to take effect. Renal replacement therapy is frequently necessary and amyloidosis now accounts for 1–2% of patients with end-stage renal failure accepted into European dialysis programmes. Renal (Pasternack *et al*, 1986) and cardiac (Hosenpud *et al*, 1991) transplantation have a role in selected cases.

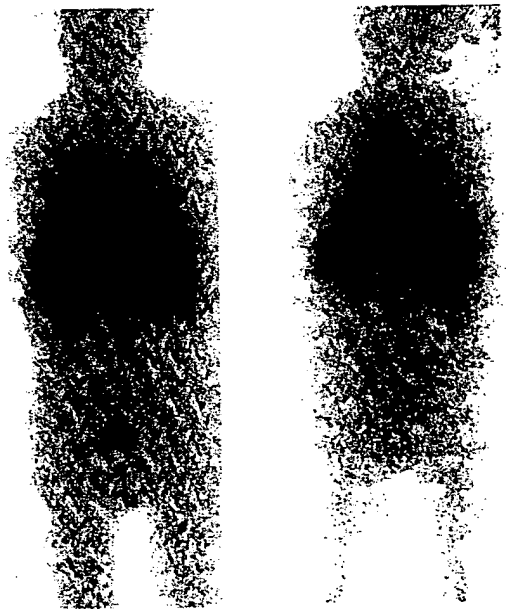

Fig 2. Serial posterior whole body ^{123}I-SAP scans in a man who presented with nephrotic syndrome and renal impairment due to systemic AL amyloidosis. The renal amyloid deposits visualized at presentation (left) had regressed substantially 3 years later (right) following 'VAD' chemotherapy, associated with recovery of normal renal function. The remainder of the images represents a normal blood-pool background signal, the level of which is inversely proportional to the whole body load of amyloid.

Prevention of amyloidosis is occasionally possible. The AA amyloid deposition associated with familial Mediterranean fever, can be inhibited by prophylactic colchicine (Goldfinger, 1972; Livneh *et al*, 1993), and dialysis-related amyloidosis is avoided by early renal transplantation (Koch, 1992).

Reactive systemic, AA amyloidosis. Without control of the acute-phase response, AA amyloidosis has a poor prognosis, with 50% and 25%, 5- and 15-year survival rates respectively, death usually being related to renal failure. The aim of therapy in systemic AA amyloidosis is to suppress the underlying disease process and reduce synthesis of serum amyloid A (SAA), from which amyloid fibrils are derived, thereby preventing further amyloid deposition.

There have been few systematic treatment studies looking at the effects of this approach. Seminal observations were made in a study of 51 patients with AA amyloidosis and JCA, who were treated with chlorambucil (Schnitzer & Ansell, 1977). All patients had proteinuria at diagnosis, but only 36% of the treated group had proteinuria after 3 years of continuous treatment. Moreover, there was 100% survival at 5 years and 68% survival at 15 years in the treated group, whereas none of the controls were alive at 15 years (David, 1991).

The only randomized, prospective treatment trial so far reported in AA amyloidosis, compared cytotoxic therapy in 11 patients with RA with 11 untreated controls (Ahlmen *et al*, 1987). End-stage renal failure developed in two patients treated with chlorambucil, cyclophosphamide or azathioprine after 54 months follow-up. In contrast, seven control patients had reached end-stage by 46 months ($P < 0{\cdot}04$). Proteinuria was not reported, but treatment with cytotoxics did result in substantial reductions in plasma SAA values. Further studies have demonstrated improved renal and actuarial survival following treatment of AA amyloidosis complicating rheumatic conditions with alkylating agents (Falck *et al*, 1979; Berglund *et al*, 1987).

SAP scintigraphy is exquisitely sensitive in AA amyloidosis, and prospective studies have confirmed that the

disease is progressive in nearly all patients with a sustained acute phase response, albeit at differing rates between individuals (Hawkins *et al*, 1993b). Amongst patients with AA amyloid, in whom the acute phase response is controlled, serial scans over a 2–3-year period have shown that the quantity of amyloid remains stable in about one-half, and regresses, again at differing rates, but often substantially, in the remainder (Hawkins *et al*, 1994b). Regression of AA amyloid has now been extensively documented in patients with RA and JCA in response to chlorambucil, with JCA that has undergone spontaneous remission, in familial Mediterranean fever in response to colchicine (Ravid *et al*, 1977), and in patients with osteomyelitis and Castleman's tumours (Vigushin *et al*, 1994a) following successful surgical excision.

Monitoring of SAA levels should therefore be an integral part of the management of all patients with AA amyloidosis (Wilkins *et al*, 1994). Successful maintainance of SAA at normal levels prevents progression of the disease, may result in regression, and often improves the prognosis dramatically.

Monoclonal immunoglobulin (AL) amyloidosis. Systemic AL amyloidosis has a far worse prognosis than AA type. In the extensive Mayo Clinic experience (Kyle & Bayrd, 1975; Kyle & Greipp, 1983), comprising over 400 cases of AL amyloidosis, median survival was only 12–15 months, and often less when associated with multiple myeloma. Approximately 50% of deaths are cardiac, and when heart failure is evident at presentation, median survival is only about 6 months (Kyle & Greipp, 1978; Kyle *et al*, 1986). Other poor prognostic factors include renal failure, jaundice and a large total body amyloid load on SAP scintigraphy.

The aim of treatment is to suppress the underlying B-cell clone, and thereby production of the amyloid fibril precursor protein, but there are many difficulties. Low-grade plasma cell dyscrasias may be less chemosensitive than multiple myeloma, and are difficult to monitor. In addition, some patients have such advanced disease at presentation that their prognosis is too short for any cytotoxic regime to be effective.

SAP scintigraphy has demonstrated that deposits in AL amyloidosis are considerably more heterogenous with respect to their anatomical distribution, quantity, rate of progression and response to treatment, than in AA type (Hawkins *et al*, 1994c). Unfortunately, response to treatment is extremely variable and a number of patients thus receive toxic treatment, with little or no apparent benefit. A management scheme for newly presenting patients with systemic AL amyloidosis is given in Fig 3.

(i) *Melphalan and prednisolone.* The only treatment regime for systemic AL amyloidosis that has been evaluated by formal clinical trials is melphalan and prednisolone. The first such trial was a placebo-controlled, double-blind study of 55 patients with AL amyloidosis (Kyle & Greipp, 1978). Among 24 nephrotic patients, treated with melphalan and prednisolone, proteinuria resolved in two and was reduced by more than 50% in eight others; renal function did not deteriorate in any of these responders. Of 13 patients who received melphalan and prednisolone for more than 12 months, clinical features of amyloidosis improved in six, remained stable in three and worsened in four. Subsequent trials have also shown benefit with melphalan and prednisolone in AL amyloidosis (Kyle *et al*, 1985). The Mayo clinic investigators reported long-term follow-up on 153 patients receiving this regime for 24–36 months, in an attempt to identify which patients derived most benefit (Gertz *et al*, 1991). Response was defined as reduction of proteinuria by at least 50%, without increase in serum creatinine, or a return to normal creatinine levels, if elevated prior to therapy. In patients with hepatic involvement, response was defined as normalization of serum alkaline phosphatase without palpable hepatomegaly, and in patients with cardiomyopathy, total resolution of congestive heart failure was required. Among patients with nephrotic syndrome, a normal serum creatinine and no evidence of cardiac amyloid, the response rate was 39% (12/31). Of 34 patients with amyloid cardiomyopathy, five responded, of whom two were alive 10 years after diagnosis. The median time to achieve a response was 11·7 months and the median survival of the 27 responders was 89·4 months. Among 126 patients who did not respond, median survival was 14·7 months.

The use of colchicine has variously been examined in AL amyloidosis, most exhaustively in a recent study of 220 patients treated with either colchicine alone, melphalan and prednisolone, or melphalan and prednisolone plus colchicine (Kyle *et al*, 1997). Median survival was 8·5 months, 17 months and 16 months respectively. Alkylating therapy with melphalan and prednisolone thus appears to be of benefit, although limited, whereas colchicine almost certainly is not, and is no longer used to treat AL amyloidosis. The duration of therapy must be balanced against the risk of myelotoxicity; at present 6-weekly cycles, for a total of 12–24 months would seem reasonable. Since response to this form of chemotherapy is often delayed for at least 12 months, early diagnosis and treatment is desirable, and may improve outcome.

(ii) *Dose-intensive chemotherapy and transplantation.* When given for multiple myeloma, the combination regime of vincristine, Adriamycin and dexamethasone (VAD), induces remission in 85% of patients with a near maximum response after two (monthly) cycles of treatment, with only modest toxicity. The VAD regime has not been subjected to a controlled trial in AL amyloidosis, but has been administered to many patients on an *ad hoc* basis. In our unit we have studied 26 patients with AL amyloidosis, treated with VAD (Persey *et al*, 1996). Among cases followed for at least 12 months, about half have shown a clinical response, defined as remission of nephrotic syndrome, reversal of renal impairment, or normalization of liver function tests. Several patients have shown substantial clinical improvement within 6 months of instituting treatment, and two patients have been in clinical remission for over 5 years. Most patients who showed clinical improvement also showed regression of amyloid on serial SAP scintigraphic studies. Two patients, both of whom had advanced cardiac amyloidosis at diagnosis, died of heart failure during treatment, and the potential cardiotoxicity of Adriamycin must be borne in mind. Other adverse effects of VAD therapy include exacerbation of neuropathy, sepsis, fluid retention and loss of bone mass.

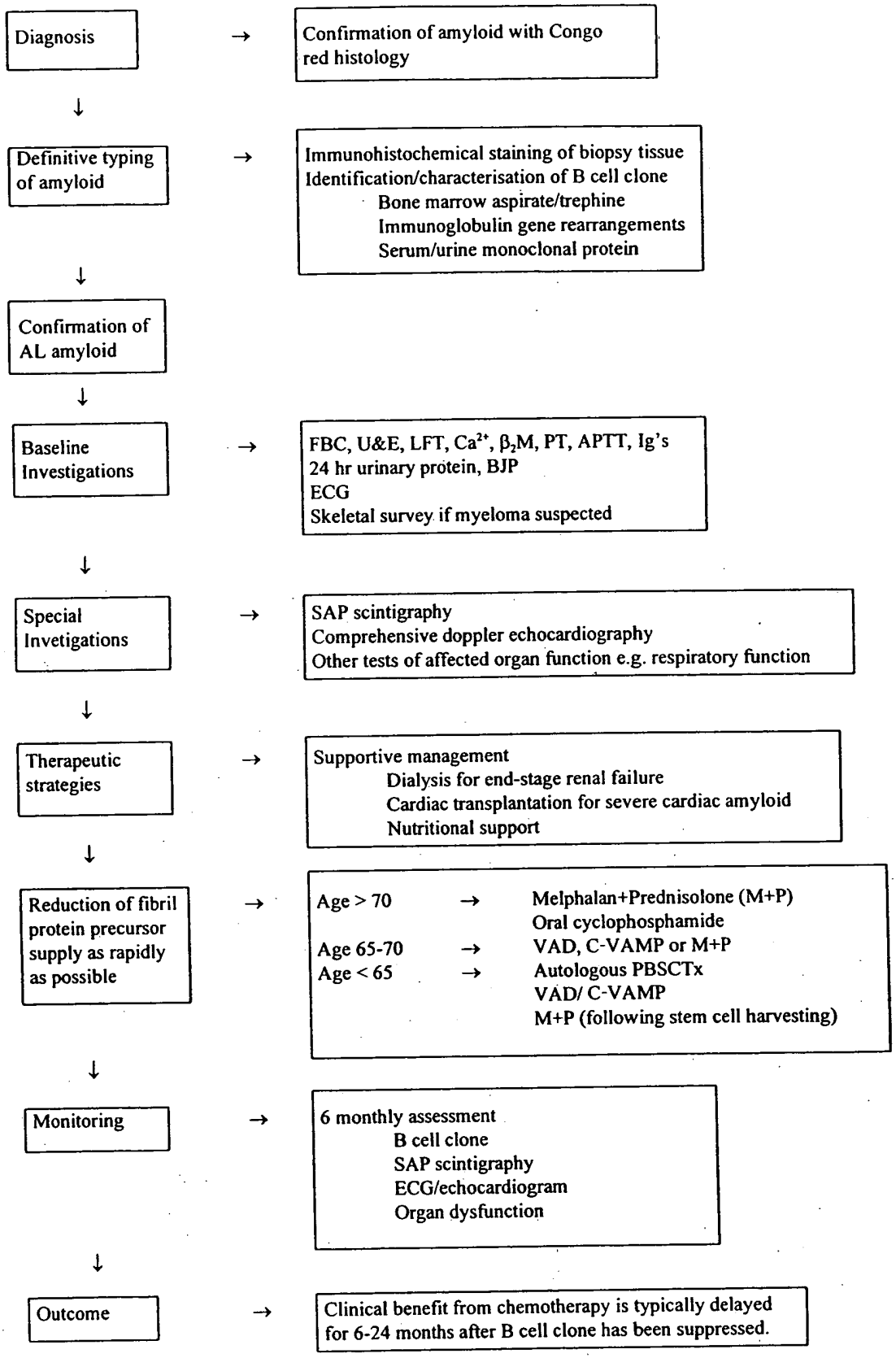

Fig 3. Scheme for management of new patients with systemic AL amyloidosis.

Single-shot high-dose chemotherapy (200 mg/m^2 melphalan) followed by periperal blood stem-cell rescue is presently undergoing open trial in Boston and such treatment of 19 patients with systemic AL amyloidosis has recently been reported (Comenzo *et al*, 1996). CD34-selected cells were used in six cases. Median time to reconstitution of neutrophils and platelets was 10 and 14 days respectively. Follow-up evaluations were at 3, 12 and 24 months post-treatment and consisted of bone marrow biopsy, immunofixation of serum and urine, and study of amyloid-related organ dysfunction. Two patients died following the procedure, one from sepsis and one from sudden cardiac death. Among the remaining 17 patients, follow-up at 3 months showed that the underlying plasma cell dyscrasia was in complete remission in 11 (65%) and amyloid-related organ dysfunction had improved in six (35%), was stable in 10 (59%) and had progressed in one (6%). Among nine patients followed up for 12 months, there was evidence of improved organ function in eight cases (89%) and deterioration in one case (11%). Sustained complete remission of the plasma cell dyscrasia and improved organ function was observed in each of the two patients who had been followed up for 24 months. Remarkably the observed clinical improvements included neuropathy.

Among the patients with systemic AL amyloidosis evaluated in our Unit, 16 have now undergone high-dose chemotherapy and autologous peripheral blood stem-cell rescue. A single 63-year-old patient died as a result of the procedure, of intestinal haemorrhage having presented with gastro-intestinal symptoms as his predominant feature. The follow-up period in our patients is insufficient for analysis of results at present. The question of melphalan dosing in patients with renal insufficiency remains difficult, but already the concept of high-dose chemotherapy with autologous peripheral blood stem-cell rescue has offered new hope for patients with systemic AL amyloidosis. We are hoping to co-ordinate a protocol for the selection and follow-up of these patients in the near future.

(iii) *Other regimes.* The effect of interferon-α2b was evaluated in an open trial at the Mayo clinic in 15 patients with AL amyloidosis who had no response to traditional cytotoxic therapy (Gertz & Kyle, 1993). None showed objective evidence of improvement, and the authors concluded that there is no place for interferon in the treatment of AL amyloidosis.

There have been reports of substantial clinical improvements following administration of the anthracycline derivative 4′-iodo-4′-deoxydoxorubicin (I-DOX) (Merlini *et al*, 1995). This agent, unlike doxorubicin (Adriamycin), binds avidly to amyloid fibrils *in vitro*, and inhibits amyloid fibril formation under experimental conditions. Disease stabilization and clinical improvement have recently been reported in eight patients with AL amyloidosis treated with I-DOX, apparently independently of cytotoxic effects. However, most of these cases had previously undergone chemotherapy and, on follow-up, five of the patients had died within 3 years. The efficacy of I-DOX is therefore not yet clear.

(iv) *Supportive care and organ transplantation.* Supportive care has an important role in the management of AL amyloidosis. Renal replacement therapy for end-stage renal failure substantially prolongs survival, particularly in the absence of associated cardiac involvement (Martinez-Vea *et al*, 1990; Gertz *et al*, 1992; Moroni *et al*, 1992). Survival in renal transplantation for end-stage renal failure due to AL amyloid compares favourably with transplantation for other causes of renal failure, including non-systemic causes (Hartmann *et al*, 1992). Recurrence of amyloid in the transplanted kidney may occur, but is rarely the cause of graft failure.

Few cardiac transplants have been performed in patients with systemic AL amyloidosis. The first reported series of 10 patients with AL amyloidosis who underwent cardiac transplantation, showed no difference in graft or patient survival at 3–4 years, compared with heart recipients from other causes (Hosenpud *et al*, 1991). At 5 years however, there was an adverse trend amongst the amyloid group. Our centre has lately reported the 10-year survival of a man who presented with terminal heart failure due to systemic AL amyloidosis, who received a cardiac transplant, followed by chemotherapy, resulting in substantial regression of his amyloid deposits, demonstrated by serial SAP scans and histology (Hall & Hawkins, 1994). In selected cases, life-saving intervention with cardiac transplantation thus provides a window of opportunity whilst other measures are instituted.

Systemic AL amyloidosis with splenic involvement is occasionally accompanied by acquired deficiency of clotting factors IX and/or X which may lead to catastrophic haemorrhage (Griepp *et al*, 1981). Splenectomy can be an effective way of correcting the clotting defect (Greipp *et al*, 1979), although there are reports of resolution of the coagulopathy following cytotoxic chemotherapy (Camoriano *et al*, 1987).

Hereditary amyloidosis. Until recently, the treatment of FAP associated with variant forms of TTR was limited to supportive measures to deal with malnutrition, bladder and bowel dysfunction, hypotension and renal and cardiac complications. However, in the knowledge that plasma TTR is essentially derived entirely from the liver, the first orthotopic liver transplant for FAP was performed in Sweden in 1991 (Holmgren *et al*, 1991). This resulted in rapid disappearance of the variant TTR from the plasma and subsequently three of the first four patients to be transplanted were reported to have marked symptomatic improvement, particularly with regard to the autonomic neuropathy (Holmgren *et al*, 1993). Although the small neural deposits are beyond the resolution of the technique, serial SAP scintigraphy has since revealed regression of visceral deposits following successful liver transplantation in many patients and this treatment is now an accepted mode of therapy for TTR amyloidosis (Hawkins *et al*, 1996). Important questions remain about the timing of transplantation, and the long-term outcome of the procedure.

Dialysis-related (β_2M) amyloid. Arthralgia due to β_2M amyloidosis may respond partially to non-steroidal anti-inflammatory drugs or corticosteroids (Bardin, 1994), but even the most severe symptoms usually disappear following successful renal transplantation (Koch, 1992).

The mechanism underlying such rapid improvement is not clear, and certainly there is no evidence that the amyloid deposits regress over so short a time. Although anti-rejection steroid therapy may have a role, symptoms of DRA continue to improve in transplant recipients even after steroids are eventually withdrawn. Also, although β_2M amyloid has been identified histologically many years after renal transplantation (Nelson *et al*, 1993), and the associated radiological bone cysts persist (Jadoul *et al*, 1989), follow-up SAP scintigraphy has shown that progression of articular amyloid is halted, and that after 5 years some regression of β_2M amyloid can be found in nearly all cases (Tan *et al*, 1996).

Conclusion

The improvement in supportive care, in conjunction with the recent advances in treatment of the systemic amyloidoses, has resulted in substantial benefit to many patients. Despite this however, new approaches to therapy are still required if the prognosis of amyloidosis is to be improved in the future. Exciting progress in understanding the molecular pathology of amyloid offers hope that such new approaches may soon become possible.

ACKNOWLEDGMENTS

The work was supported by Project and Programme grants from the Medical Research Council. We thank Beth Sontrop for expert preparation of the manuscript.

Immunological Medicine Unit,
Department of Medicine,
Royal Postgraduate Medical School,
Hammersmith Hospital, London

JULIAN D. GILLMORE
PHILIP N. HAWKINS
MARK B. PEPYS

REFERENCES

Ahlmen, M., Ahlmen, J., Svalander, C. & Bucht, H. (1987) Cytotoxic drug treatment of reactive amyloidosis in rheumatoid arthritis with special reference to renal insufficiency. *Clinical Rheumatology*, 6, 27–38.

Axelrad, M.A., Kisilevsky, R., Willmer, J., Chen, S.J. & Skinner, M. (1982) Further characterisation of amyloid-enhancing factor. *Laboratory Investigation*, 47, 139–146.

Baltz, M.L., Caspi, D., Evans, D.J., Rowe, I.F., Hind, C.R.K. & Pepys, M.B. (1986) Circulating serum amyloid P component is the precursor of amyloid P component in tissue amyloid deposits. *Clinical and Experimental Immunology*, 66, 691–700.

Bardin, T. (1994) Low-dose prednisone in dialysis-related amyloid arthropathy. *Revue du Rhumatisme [English Edition]*, 61, (Suppl.), 97S–100S.

Benson, M.D., Liepnieks, J., Uemichi, T., Wheeler, G. & Correa, R. (1993) Hereditary renal amyloidosis associated with a mutant fibrinogen α-chain. *Nature Genetics*, 3, 252–255.

Benson, M.D. & Uemichi, T. (1996) Transthyretin amyloidosis. *Amyloid: The International Journal of Experimental and Clinical Investigations*, 3, 44–56.

Berglund, K., Keller, C. & Thysell, H. (1987) Alkylating cytostatic treatment in renal amyloidosis secondary to rheumatic disease. *Annals of the Rheumatic Diseases*, 46, 757–762.

Booth, D., Sunde, M., Bellotti, V., Robinson, C., Hutchinson, L., Fraser, P., Hawkins, P., Dobson, C., Radford, S., Blake, C. & Pepys, M. (1997) Instability, unfolding, and aggregation of human lysozyme variants underlying amyloid fibrillogenesis. *Nature*, 385, 787–793.

Camoriano, J., Greipp, P., Bayer, G. & Bowie, E. (1987) Resolution of acquired factor X deficiency and amyloidosis with melphalan and prednisone therapy. *New England Journal of Medicine*, 316, 1133–1135.

Comenzo, R.L., Sarnacki, D., Finn, K., Falk, R., Sanchorawala, V., Akpek, G., Farhat, M. & Vosburgh, E. (1996) AL amyloidosis responds to high-dose melphalan and blood stem cells. (Abstract). *Blood*, 88, (Suppl. 1), 686a.

Costa, P.P., Figueira, A.S. & Bravo, F.R. (1978) Amyloid fibril protein related to prealbumin in familial amyloidotic polyneuropathy. *Proceedings of the National Academy of Sciences of the United States of America*, 75, 4499–4503.

David, J. (1991) Amyloidosis in juvenile chronic arthritis. *Clinical and Experimental Rheumatology*, 9, 73–78.

Delgado, A.W. & Mosqueda, A. (1989) A highly sensitive method for diagnosis of secondary amyloidosis by labial salivary gland biopsy. *Journal of Oral Pathology and Medicine*, 18, 310–314.

Duchen, L.W. (1992) Current status review: cerebral amyloid. *International Journal of Experimental Pathology*, 73, 535–550.

Falck, H.M., Törnroth, T., Skrifvars, B. & Wegelius, O. (1979) Resolution of renal amyloidosis secondary to rheumatoid arthritis. *Acta Medica Scandinavica*, 205, 651–656.

Gagnon, R.F., Bourgouin, P., Lough, J., Somerville, P. & Kaye, M. (1986) Carpal tunnel syndrome with beta 2-microglobulin-containing amyloid and continuous ambulatory peritoneal dialysis. *American Journal of Kidney Diseases*, 8, A6.

Gejyo, F., Yamada, T., Odani, S., Nakogawa, Y., Arakawa, M., Kimitomo, T., Kataoka, H., Suzuki, M., Hirasawa, Y., Shirahama, T., Cohen, A.S. & Schmid, K. (1985) A new form of amyloid protein associated with chronic hemodialysis was identified as β_2-microglobulin. *Biochemical and Biophysical Research Communications*, 129, 701–706.

Gertz, M. & Kyle, R. (1993) Phase II trial of recombinant interferon alfa-2 in the treatment of primary systemic amyloidosis. *American Journal of Hematology*, 44, 125–128.

Gertz, M.A. & Kyle, R.A. (1991) Secondary systemic amyloidosis: response and survival in 64 patients. *Medicine*, 70, 246–256.

Gertz, M.A., Kyle, R.A. & Greipp, P.R. (1991) Response rates and survival in primary systemic amyloidosis. *Blood*, 77, 257–262.

Gertz, M.A., Kyle, R.A. & O'Fallon, W.M. (1992) Dialysis support of patients with primary systemic amyloidosis: a study of 211 patients. *Archives of Internal Medicine*, 152, 2245–2250.

Glenner, G.G. (1980a) Amyloid deposits and amyloidosis: the β-fibrilloses. I. *New England Journal of Medicine*, 302, 1283–1292.

Glenner, G.G. (1980b) Amyloid deposits and amyloidosis: the β-fibrilloses. II. *New England Journal of Medicine*, 302, 1333–1343.

Goldfinger, W.E. (1972) Colchicine for familial Mediterranean fever. *New England Journal of Medicine*, 287, 1302.

Gorevic, P.D., Munoz, P.C., Casey, T.T., DiRaimondo, C.R., Stone, W.J., Prelli, F.C., Rodriques, M.M., Ponlik, M.D. & Frangione, B. (1986) Polymerization of intact β_2-microglobulin in tissues causes amyloidosis in patients on chronic hemodialysis. *Proceedings of the National Academy of Sciences of the United States of America*, 83, 7908–7912.

Greipp, P.R., Kyle, R.A. & Bowie, E.J. (1979) Factor X deficiency in primary amyloidosis: resolution after splenectomy. *New England Journal of Medicine*, 301, 1050–1051.

Griepp, P.R., Kyle, R.A. & Bowie, E.J.W. (1981) Factor X deficiency in amyloidosis: a critical review. *American Journal of Hematology*, 11, 443–450.

Hall, R. & Hawkins, P.N. (1994) Cardiac transplantation for AL amyloidosis. *British Medical Journal*, 309, 1135–1137.

Hartmann, A., Holdaas, H., Fauchald, P., Nordal, K.P., Berg, K.J. & Talseth, T. (1992) Fifteen years' experience with renal transplantation in systemic amyloidosis. *Transplant International*, 5, 15–18.

Hawkins, P.N., Hall, M., Hall, R., Penny, L., Henderson, A., Yacoub, M., Mitchell, A. & Pepys, M.B. (1994a) Regression of AL amyloidoisis and prolonged survival following cardiac transplantation and chemotherapy. *Amyloid and Amyloidosis 1993* (ed. by R. Kisilevsky, M. D. Benson, B. Frangione, J. Gauldie, T. J. Muckle and I. D. Young), pp. 657–659. Parthenon Publishing, Pearl River, New York.

Hawkins, P.N., Lavender, J.P. & Pepys, M.B. (1990) Evaluation of systemic amyloidosis by scintigraphy with ^{123}I-labelled serum amyloid P component. *New England Journal of Medicine*, 323, 508–513.

Hawkins, P.N., Myers, M.J., Epenetos, A.A., Caspi, D. & Pepys, M.B. (1988a) Specific localization and imaging of amyloid deposits *in vivo* using ^{123}I-labeled serum amyloid P component. *Journal of Experimental Medicine*, 167, 903–913.

Hawkins, P.N., Myers, M.J., Lavender, J.P. & Pepys, M.B. (1988b) Diagnostic radionuclide imaging of amyloid: biological targeting by circulating human serum amyloid P component. *Lancet*, i, 1413–1418.

Hawkins, P.N., Persey, M.R., Lovat, L.B., Madhoo, S., McCarthy, M., Heaton, N., Williams, R. & Pepys, M.B. (1996) UK experience of liver transplantation in FAP. *Neuromuscular Disorders*, 6, S77.

Hawkins, P.N., Peters, A.M., Vigushin, D.M., Richardson, S., Seymour, A., Holmgren, G., Steen, L., Woo, P., Hall, A. & Pepys, M.B. (1993a) SAP scintigraphy and turnover studies demonstrate regression of amyloidosis. *Nuclear Medicine Communications*, 14, 259–260.

Hawkins, P.N., Richardson, S., Vigushin, D.M., David, J., Kelsey, C.R., Gray, R.E.S., Hall, M.A., Woo, P., Lavender, J.P. & Pepys, M.B. (1993b) Serum amyloid P component scintigraphy and turnover studies for diagnosis and quantitative monitoring of AA amyloidosis in juvenile rheumatoid arthritis. *Arthritis and Rheumatism*, 36, 842–851.

Hawkins, P.N., Vigushin, D.M., Richardson, S., Seymour, A., Holmgren, G., Steen, L., Woo, P., Hall, A., Peters, A.M. & Pepys, M.B. (1994b) Natural history and regression of amyloidosis. *Amyloid and Amyloidosis 1993* (ed. by R. Kisilevsky, M. D. Benson, B. Frangione, J. Gauldie, T. J. Muckle and I. D. Young), pp. 638–641. Parthenon Publishing, Pearl River, New York.

Hawkins, P.N., Vigushin, D.M., Richardson, S., Seymour, A. & Pepys, M.B. (1994c) Evaluation of 100 cases of systemic AL amyloidosis by serum amyloid P component (SAP) scintigraphy. *Amyloid and Amyloidosis 1993* (ed. by R. Kisilevsky, M. D. Benson, B. Frangione, J. Gauldie, T. J. Muckle and I. D. Young), pp. 209–211. Parthenon Publishing, Pearl River, New York.

Holmgren, G., Ericzon, B.-G., Groth, C.-G., Steen, L., Suhr, O., Andersen, O., Wallin, B.G., Seymour, A., Richardson, S., Hawkins, P.N. & Pepys, M.B. (1993) Clinical improvement and amyloid regression after liver transplantation in hereditary transthyretin amyloidosis. *Lancet*, 341, 1113–1116.

Holmgren, G., Steen, L., Ekstedt, J., Groth, C.-G., Ericzon, B.-G., Eriksson, S., Andersen, O., Karlberg, I., Norden, G., Nakazato, M., Hawkins, P., Richarson, S. & Pepys, M. (1991) Biochemical effect of liver transplantation in two Swedish patients with familial amyloidotic polyneuropathy (FAP-met^{30}). *Clinical Genetics*, 40, 242–246.

Hosenpud, J.D., DeMarco, T., Frazier, O.H., Griffith, B.P., Uretsky, B.F., Menkis, A.H., O'Connell, J.B., Olivari, M.-T. & Valantine, H.A. (1991) Progression of systemic disease and reduced long-term survival in patients with cardiac amyloidosis undergoing heart transplantation. *Circulation*, 84, 338–343.

Husby, G. (1992) Nomenclature and classification of amyloid and amyloidoses. *Journal of Internal Medicine*, 232, 511–512.

Jadoul, M., Malghem, J., Pirson, Y., Maldague, B. & van Ypersele de Strihou, C. (1989) Effect of renal transplantation on the radiological signs of dialysis amyloid osteoarthropathy. *Clinical Nephrology*, 32, 194–197.

Koch, K.M. (1992) Dialysis-related amyloidosis. (Clinical conference). *Kidney International*, 41, 1416–1429.

Kyle, R.A. & Bayrd, E.D. (1975) Amyloidosis: a review of 236 cases. *Medicine*, 54, 271–299.

Kyle, R., Gertz, M., Greipp, P., Witzig, T., Lust, J., Lacy, M. & Therneau, T. (1997) A trial of three regimes for primary amyloidosis: colchicine alone, melphalan and prednisolone, and melphalan, prednisolone and colchicine. *New England Journal of Medicine*, 336, 1202–1207.

Kyle, R.A. & Greipp, P.R. (1978) Primary systemic amyloidosis: comparison of melphalan and prednisone versus placebo. *Blood*, 52, 818–827.

Kyle, R.A. & Greipp, P.R. (1983) Amyloidosis (AL): clinical and laboratory features in 229 cases. *Mayo Clinic Proceedings*, 58, 665–683.

Kyle, R.A., Greipp, P.R., Garton, J.P. & Gertz, M.A. (1985) Primary systemic amyloidosis: comparison of melphalan/prednisone versus colchicine. *American Journal of Medicine*, 79, 708–716.

Kyle, R.A., Greipp, P.R. & O'Fallon, M. (1986) Primary systemic amyloidosis: multivariate anlysis for prognostic factors in 168 cases. *Blood*, 68, 220–224.

Livneh, A., Zemer, D., Langevitz, P., Shemer, J., Sohar, E. & Pras, M. (1993) Colchicine in the treatment of AA and AL amyloidosis. *Seminars in Arthritis and Rheumatism*, 23, 206–214.

Lorenzo, A., Razzaboni, B., Weir, G.C. & Yankner, B.A. (1994) Pancreatic islet cell toxicity of amylin associated with type-2 diabetes mellitus. *Nature*, 368, 756–760.

Martinez-Vea, A., Garcia, C., Carreras, M., Revert, L. & Oliver, J.A. (1990) End-stage renal disease in systemic amyloidosis: clinical course and outcome on dialysis. *American Journal of Nephrology*, 10, 283–289.

Maury, C.P.J. (1991) Gelsolin-related amyloidosis: identification of the amyloid protein in Finnish hereditary amyloidosis as a fragment of variant gelsolin. *Journal of Clinical Investigation*, 87, 1195–1199.

Merlini, G., Ascari, E., Amboldi, N., Bellotti, V., Arbustini, E., Perfetti, V., Ferrari, M., Zorzoli, I., Marinone, M., Garini, P., Diegoli, M., Trizio, D. & Ballinari, D. (1995) Interaction of the anthracycline 4′-iodo-4′-deoxydoxorubicin with amyloid fibrils: inhibition of fibrillogenesis. *Proceedings of the National Academy of Sciences of the United States of America*, 92, 2959–2963.

Moroni, G., Banfi, G., Montoli, A., Bucci, A., Bertani, T., Ravelli, M., Pozzi, C., Leonelli, M., Lupo, A., Volpi, A., Pasquali, S. & Salvadeo, A. (1992) Chronic dialysis in patients with systemic amyloidosis: the experience in Northern Italy. *Clinical Nephrology*, 38, 81–85.

Nelson, S., Sharpestone, P. & Kingswood, J. (1993) Does dialysis-related amyloidosis resolve after transplantation? *Nephrology, Dialysis, Transplantation*, 8, 369–370.

Nelson, S.R., Lyon, M., Gallagher, J.T., Johnson, E.A. & Pepys, M.B. (1991) Isolation and characterization of the integral glycosaminoglycan constituents of human amyloid A and monoclonal light-chain amyloid fibrils. *Biochemical Journal*, 275, 67–73.

Nichols, W.C., Gregg, R.E., Brewer, H.B.J. & Benson, M.D. (1990) A mutation in apolipoprotein A-I in the Iowa type of familial amyloidotic polyneuropathy. *Genomics*, 8, 318–323.

Pasternack, A., Ahonen, J. & Kuhlback, B. (1986) Renal transplantation in 45 patients with amyloidosis. *Transplantation*, 42, 598–601.

Pepys, M.B. (1995) Amyloidosis. *Oxford Textbook of Medicine*, Vol. 2. 3rd edn (ed. by D. J. Weatherall, J. G. G. Ledingham and D. A. Warrell), pp. 1512–1524. Oxford University Press.

Pepys, M.B., Hawkins, P.N., Booth, D.R., Vigushin, D.M., Tennent, G.A., Soutar, A.K., Totty, N., Nguyen, O., Blake, C.C.F., Terry, C.J., Feest, T.G., Zalin, A.M. & Hsuan, J.J. (1993) Human lysozyme gene mutations cause hereditary systemic amyloidosis. *Nature*, 362, 553–557.

Pepys, M.B., Rademacher, T.W., Amatayakul-Chantler, S., Williams, P., Noble, G.E., Hutchinson, W.L., Hawkins, P.N., Nelson, S.R., Gallimore, J.R., Herbert, J., Hutton, T. & Dwek, R.A. (1994) Human serum amyloid P component is an invariant constituent of amyloid deposits and has a uniquely homogeneous glycostructure. *Proceedings of the National Academy of Sciences of the United States of America*, 91, 5602–5606.

Persey, M.R., Lovat, L.B., Apperley, J.F., Madhoo, S., Pepys, M.B. & Hawkins, P.N. (1996) Intensive chemotherapy for AL amyloidosis. *British Journal of Rheumatology*, 35, (Suppl. 1), 12.

Puchtler, H., Sweat, F. & Levine, M. (1962) On the binding of Congo red by amyloid. *Journal of Histochemistry and Cytochemistry*, 10, 355–364.

Ravid, M., Shapira, J. & Kedar, I. (1977) Prolonged colchicine treatment in four patients with amyloidosis. *Annals of Internal Medicine*, 87, 568–570.

Saraiva, M.J.M., Birken, S., Costa, P.P. & Goodman, D.S. (1984) Amyloid fibril protein in familial amyloid polyneuropathy, Portuguese type: definition of molecular abnormality in transthyretin (prealbumin). *Journal of Clinical Investigation*, 74, 104–119.

Scheuner, D., Eckman, C., Jensen, M., Song, X., Citron, M., Suzuki, N., Bird, T., Hardy, J., Hutton, M., Kukull, W., Larson, E., Levy-Lahad, E., Viitanen, M., Peskind, E., Poorkaj, P., Schellenberg, G., Tanzi, R., Wasco, W., Lannfelt, L., Selkoe, D. & Younkin, S. (1996) Secreted amyloid beta-protein similar to that in the senile plaques of Alzheimer's disease is increased *in vivo* by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. *Nature Medicine*, 2, 864–870.

Schnitzer, T.J. & Ansell, B.M. (1977) Amyloidosis in juvenile chronic polyarthritis. *Arthritis and Rheumatism*, 20, 245–252.

Soutar, A.K., Hawkins, P.N., Vigushin, D.M., Tennent, G.A., Booth, S.E., Hutton, T., Nguyen, O., Totty, N.F., Feest, T.G., Hsuan, J.J. & Pepys, M.B. (1992) Apolipoprotein AI mutation Arg-60 causes autosomal dominant amyloidosis. *Proceedings of the National Academy of Sciences of the United States of America*, 89, 7389–7393.

Tan, S.-Y., Irish, A., Winearls, C.G., Brown, E.A., Gower, P.E., Clutterbuck, E.J., Madhoo, S., Lavender, J.P., Pepys, M.B. & Hawkins, P.N. (1996) Long term effect of renal transplantation on dialysis-related amyloid deposits and symptomatology. *Kidney International*, 50, 282–289.

Tan, S.-Y., Pepys, M.B. & Hawkins, P.N. (1995) Treatment of amyloidosis. *American Journal of Kidney Diseases*, 26, 267–285.

Vigushin, D.M., Pepys, M.B. & Hawkins, P.N. (1994a) Rapid regression of AA amyloidosis following surgery for Castleman's disease. *Amyloid and Amyloidosis 1993* (ed. by R. Kisilevsky, M. D. Benson, B. Frangione, J. Gauldie, T. J. Muckle and I. D. Young), pp. 48–50. Parthenon Publishing, Pearl River, New York.

Vigushin, D.M., Vulliamy, T., Kaeba, J.S., Hawkins, P.N., Luzzatto, L. & Pepys, M.B. (1994b) Immunoglobulin gene rearrangement analysis in AL amyloidosis. *Amyloid and Amyloidosis 1993* (ed. by R. Kisilevsky, M. D. Benson, B. Frangione, J. Gauldie, T. J. Muckle and I. D. Young), pp. 287–289. Parthenon Publishing, Pearl River, New York.

Westermark, P. & Stenkvist, B. (1973) A new method for the diagnosis of systemic amyloidosis. *Archives of Internal Medicine*, 132, 522–523.

Wilkins, J., Gallimore, J.R., Tennent, G.A., Hawkins, P.N., Limburg, P.C., van Rijswijk, M.H., Moore, E.G. & Pepys, M.B. (1994) Rapid automated enzyme immunoassay of serum amyloid A. *Clinical Chemistry*, 40, 1284–1290.

Wisniewski, K., Wisniswski, H. & Wen, G. (1985) Occurrence of neuropathological changes and dementia of Alzheimer's disease in Down's syndrome. *Annals of Neurology*, 17, 278–282.

Keywords: amyloid, amyloidosis, SAP, scintigraphy.